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action of Epac on the global [Ca2+]i transient, Epac induced marked increase of [Ca2+]n. These effects were abolished by knocking down Epac1 expression (adenovirus ShEpac1 infection), indicating that the 8-p-CPT effects involved Epac1 activation. Moreover, the Epac-dependent effects on Ca2+ handling were prevented by the calmodulin kinase type II inhibitor, KN93, and the inositol triphosphate receptor blocker, 2APB. We conclude that Epac effects on Ca2+ handling are compartmentalized.

**436-Pos Board B236**

**Isoflurane Increases Mitochondrial Free $Ca^{2+}$ by Enhancing Transport via the $Ca^{2+}$ Uniporter Independent of $\Delta\Psi_m$**

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Modulation of mitochondrial free $Ca^{2+}$ (m[$Ca^{2+}$]) is implicated as one of the possible upstream factors that initiates anesthetic-mediated cardioprotection against ischemia-reperfusion (IR) injury. To help unravel the mechanisms by which volatile anesthetics modulate m[$Ca^{2+}$], experiments were conducted to spectrofluorometrically measure dose-dependent effects of isoflurane (0.5-2 mM) on the time courses of mitochondrial bioenergetics (NADH redox state, respiration, and $\Delta\Psi_m$) and $Ca^{2+}$ uptake into the matrix. Isolated mitochondria from rat hearts were energized with 10 mM pyruvate/malate (state 2); this was followed by sequentially adding isoflurane (0.5 to 2 mM), 0.5 mM $CaCl_2$ (with 1 mM EGTA in the buffer),and 250 μM ADP (state 3). The data showed that: (a) isoflurane dose-dependently increased m[$Ca^{2+}$] in state 2 in spite of a slight $\Delta\Psi_m$ depolarization, (b) isoflurane increased the duration of state 3 respiration as well as the duration of the ADP-induced rise in m[$Ca^{2+}$], and (c) isoflurane decreased state 3 NADH oxidation (i.e., increased NADH level compared to control). These data indicate the possible roles of isoflurane (1) to modulate m[$Ca^{2+}$] by directly activating the $Ca^{2+}$ uniporter, independent of $\Delta\Psi_m$, (2) to decrease the rate of ADP phosphorylation while prolonging the duration of state 3 respiration (possibly by inhibiting complex I), and (3) to prolong the duration of ADP-induced increase in m[$Ca^{2+}$] response during state 3 because of reduced electron flux and slower ADP phosphorylation.

**437-Pos Board B237**

**Assesment of an Oxidant-Based Strategy to Target Cancer Cells**

**Tetyana I. Zhelay**, Shadi Zahedi, David R. Giovannucci.

Many cancer cell lines concentrate vitamin C, and in combination with vitamin K3 generate a redox-cycling that induces over-production of ROS, thus this treatment provides an oxidant challenge to cancer cells that spares non-cancer cells (Verrax et al, 2009). Because mitochondria are targets of oxidative damage by ROS, Vit K3/C treatment was predicted to induce mitochondrial dysfunction, subsequent $Ca^{2+}$ homeostasis dysregulation and cell death. However, effectiveness of this treatment for breast and neuroendocrine cancer cell lines has not been investigated. Thus, we compared mitochondrial function, $Ca^{2+}$ dynamics and cell viability prior to and following Vit K3/C treatment in human neuroendocrine and breast cancer cell lines. $Ca^{2+}$-, $\psi_m$- and ROS-sensitive dyes were used to measure mitochondrial mass, energy state and changes $Ca^{2+}$ dynamics. This study indicated that mitochondrial membrane potential was reduced in both carcinoid and breast cancer cell lines after VitK3/C treatment. Mitochondrial superoxide levels were significantly increased only in breast cancer cell lines after VitK3/C treatment and were correlated with cell death. In contrast, mitochondrial dysregulation was more effective at altering $Ca^{2+}$ signaling in neuroendocrine than in breast cancer cell lines. Treatment with Vit K3/C significantly reduced $Ca^{2+}$ entry and diminished the frequency and maintenance of $Ca^{2+}$ oscillations. Although Vit K3/C treatment was found to be generally toxic to both breast and neuroendocrine cancer cell lines, it was less effective at inducing cell death in neuroendocrine cancer cells. Thus, these data indicate that the oxidative treatment regimen may not be universally effective for all types of cancers. Moreover, the mechanism of toxicity appears to be associated with a direct oxidant challenge to mitochondria. Further study will determine if this treatment approach alone or in combination with other therapies will be a useful strategy to combat cancer in patients.

**438-Pos Board B238**

**Abnormal Sodium Handling and Mitochondrial Metabolism in Cardiac Dystrophy**

**Eva Poláková**, Natalia Shirokova.

Lack of dystrophin in striated muscle results in the activation of several stretch-induced transplasmalemmal ion influx pathways. Recently we demonstrated that mechanical challenges produce substantial inward currents in dystrophic (*mdx*) but not in WT cardiomyocytes. We also detected a significant increase in cytosolic [$Na^+$] following stretch in *mdx* cells. We suggested that beat-to-beat mechanical activity of dystrophic heart might lead to accumulation of $Na^+$ inside the cardiomyocytes ($Na^+$ overload). Here we measured resting $[Na^+]_i$ in *mdx* and WT cells using the ratiometric $Na^+$ indicator SBFI. The averaged value of $[Na^+]_i$ was indeed significantly greater in *mdx* than in WT myocytes (24.2 ± 3.1, n=13 *vs.* 14.0 ± 1.7 mM, n=9). $Na^+$ overload can have a profound effect on cellular functions. E.g. it can change the reversal potential of $NCX_{sl}$, reducing its ability to remove $Ca^{2+}$. This is in agreement with our recent report that the reverse mode of $NCX_{sl}$ contributes to cytosolic $Ca^{2+}$ overload following mechanical stress, despite little change in the resting potential. Elevated $[Na^+]_i$ can also eventually affect mitochondrial metabolism as it could enhance $Ca^{2+}$ extrusion from the mitochondria *via* $NCX_{mt}$. Therefore we measured NADH autofluorescence. Maximal oxidation of NADH by FCCP/oligomycin was taken as 0% reduced NADH, whereas maximal reduction by rotenone and β-hydroxy-butyrate was defined as 100% reduced NADH. The averaged values show that mitochondrial matrix is significantly more oxidized in resting *mdx* myocytes compared with WT cells (35% ±3.1, n=27 *vs.* 53 ± 5.2 %, NADH reduction n=10). The oxidation of mitochondrial matrix can contribute to the cellular oxidative stress and favor the opening of mPTP and cell death -observations that we previously reported for dystrophic cardiomyocytes. Taken together, our findings suggest that elevated $[Na^+]_i$ may contribute to the development of dystrophic cardiomyopathy.

**439-Pos Board B239**

**Nadph Oxidase-Stimulated Mitochondrial Radical Release Contributes to Arrhythmic Toxicity of Cardiac Glycosides by Redox Modification of Ryanodine Receptor**

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The therapeutic utility of cardiac glycosides (CGs), agents commonly used in treating heart failure (HF), is limited by arrhythmic toxicity. The adverse effects of CGs have been attributed to excessive accumulation of intracellular Ca resulting from inhibition of Na+/K+-ATPase ion transport activity. However, CGs are also known to increase intracellular reactive oxygen species (ROS), which could contribute to arrhythmogenesis through redox modification of cardiac ryanodine receptors (RyR2s). Here we sought to determine whether modification of RyR2s by ROS contributes to CG-dependent arrhythmogenesis and define the relevant signaling pathways. In isolated rat ventricular myocytes, the CG, digitoxin (DGT), increased the incidents of arrhythmogenic spontaneous Ca waves, decreased the sarcoplasmic reticulum (SR) Ca load, and increased both ROS and RyR2 thiol oxidation. Additionally, pretreatment with DGT increased spark frequency for a given SR Ca load in permeabilized myocytes. These effects on Ca waves and sparks were prevented by the antioxidant 2-mercaptopropionylglycine. The CG-dependent increases in ROS, RyR2 oxidation and arrhythmogenic propensity were prevented by inhibition of NADPH oxidase and of mitochondrial ATP-dependent K+ channels but not by inhibition of xanthine oxidase. Additionally, the frequency of DGT-dependent Ca waves was blunted by inhibition of the upstream of NADPH oxidase intracellular ROS signaling components: Src kinase, PI3K, and PKC. These results suggest that the arrhythmogenic adverse effects of CGs involve alterations in RyR2 function caused by oxidative changes in the channel structure by ROS. The CG-dependent increase in ROS may be mediated by stimulation of NADPH oxidase, via Src kinase and PI3K activation, resulting in mitochondrial radical release.

**440-Pos Board B240**

**Actions of the NAADP Antagonist Trans-Ned19 in Cardiac Ventricular Myocytes**

Laura Elson, Grant Churchill, **Derek A. Terrar**, Stevan Rakovic.

Nicotinic acid adenine dinucleotide phosphate (NAADP) is a substance that promotes calcium release from acidic intracellular calcium stores and exerts effects on calcium transients and contractions in guinea-pig ventricular myocytes. A structural analogue of NAADP, termed NED19, acts as an antagonist of NAADP in cell homogenates from sea-urchin eggs. The aim of the present study was to investigate actions of NED19 (using the trans optical isomer) on ventricular myocytes isolated from guinea-pig hearts.

Trans-NED19 (1 μM, 5 min exposure) was without significant effect on L-type calcium currents over the range −30 to +60 mV (switched voltage-clamp, step depolarizations from −40 mV). The acetoxymethylester of NAADP (NAADP-AM) was applied to ventricular myocytes with the aim of allowing permeation of NAADP-AM across the cell membrane followed by accumulation of cytosolic NAADP after de-esterification of NAADP-AM by intracellular esterases. NAADP-AM (60 nM) increased myocyte contraction by approximately 40% (n=10, field stimulation at 1 Hz). This effect of NAADP-AM was no longer observed in myocytes exposed to trans-NED19 (1 μM), consistent with an antagonist effect of trans-NED19 preventing the effects of cytosolic NAADP. Exposure of myocytes to trans-NED19 (1 μM) alone significantly reduced contraction by 17 +/- 4%, a reduction which is similar to the 20 +/- 4% previously